

What is claimed is:

1. A method of producing multiple homologous or complementary copies of a target nucleic acid sequence comprising:

5 incubating a mixture consisting essentially of:
a sample comprising said target nucleic acid sequence;
a first oligonucleotide comprising a first primer or a first promoter-primer comprising a sequence able to hybridize at or near the 3'-end of said target nucleic acid
10 sequence;

a second oligonucleotide comprising a second primer or a second promoter-primer comprising a nucleic acid sequence able to hybridize at or near the 3'-end of a complement of said target nucleic acid sequence;

15 wherein at least one of said first oligonucleotide and said second oligonucleotide comprises a promoter-primer, and separately one comprises a modified oligonucleotide or a mixture of modified and unmodified oligonucleotides able to hybridize to the same strand of said target nucleic acid
20 or its complement; wherein said modified oligonucleotide is modified to reduce or block extension of said oligonucleotide by a polymerase compared to an unmodified oligonucleotide;

one or more DNA and/or RNA dependent DNA polymerases;
25 and

an RNA polymerase able to recognize a promoter within one or both of said first or second promoter-primers,
under conditions in which a first
oligonucleotide/target sequence complex is formed and DNA
30 and RNA synthesis occurs.

2. The method of claim 1 wherein said target is RNA.

3. The method of claim 1 wherein said target is DNA and wherein said first primer hybridizes to said target distant from the 3' end of nucleic acid comprising said target.

5 4. The method of claim 1 wherein said incubating is done in the presence of an RNase H activity.

5. The method of claim 2 wherein said RNase H activity is supplied from an exogenous RNase H.

10 6. The method of claim 5 wherein said exogenous RNase H is from E. coli.

7. The method of claim 1 wherein said mixture of modified and unmodified oligonucleotides comprises a mixture of oligonucleotides with different modifications at their 3'-ends.

15 8. The method of claim 1 wherein said first oligonucleotide and said second oligonucleotide both comprise a promoter-primer.

9. The method of claim 1 or 8 wherein said first oligonucleotide and said second oligonucleotide each
20 consist essentially of a mixture of modified and unmodified oligonucleotides.

10. , The method of claim 1 wherein said mixture of modified and unmodified oligonucleotides has modifications comprising one or more of an alkane diol modification, or
25 addition of a 3' deoxynucleotide residue, one or more ribonucleotides, a nucleotide with nonphosphodiester

linkage, a non-nucleotide modification, one or more bases noncomplementary to the target, or a dideoxynucleotide.

11. The method of claim 10 wherein said mixture of modified and unmodified oligonucleotides has modifications
5 comprising one or more of an alkane diol modification, or addition of a cordycepin, a ribonucleotide, a phosphorothioate nucleotide, a non-nucleotide modification, or a dideoxynucleotide.

12. The method of claim 1 wherein a reverse
10 transcriptase comprises said DNA-dependent DNA polymerase and said RNA-dependent DNA polymerase.

13. The method of claim 12 wherein said reverse transcriptase further comprises RNase H activity.

14. The method of claim 12 or 13 wherein said reverse
15 transcriptase is MMLV reverse transcriptase or AMV reverse transcriptase.

15. The method of claim 1 wherein said RNA polymerase is selected from the group consisting of bacteriophage T7, T3, and SP6 RNA polymerase.

20 16. The method of claim 1 further comprising an assay to indicate the presence of said target nucleic acid sequence.

17. The method of claim 1 or 16 wherein said method
25 is performed in the presence of one or more helper oligonucleotides.

18. The method of claim 1 wherein said incubating is performed in the presence of one or more of DMSO, dimethylformamide, ethylene glycol, glycerol and zinc.

19. The method of claim 1 wherein said method is performed at essentially constant temperature.

20. The method of claim 1 wherein said modified and unmodified oligonucleotides are present in a ratio of 1:1 to 1000:1.

21. The method of claim 1 consisting essentially of the steps of claim 1.

22. The method of claim 2 wherein a DNA- and RNA-dependent DNA polymerase are provided.

23. The method of claim 1 wherein a third oligonucleotide comprising a sequence able to hybridize to the 5' end of an RNA target is provided to define said 5' end of target to be amplified.

24. A composition consisting essentially of:
sample comprising a target nucleic acid sequence, a first and a second oligonucleotide of opposite sense, one of said first or second oligonucleotides being able to hybridize at or near the 3'-end of said target nucleic acid sequence and the other of said first or second oligonucleotides being able to hybridize at or near a 3'-end of a nucleic acid sequence complementary to said target nucleic acid sequence, wherein one of said first or second oligonucleotides comprises a first promoter-primer and consists essentially of a single nucleic acid sequence

having both modified and unmodified members, wherein said modified oligonucleotide is modified to reduce extension of said oligonucleotide by a polymerase compared to an unmodified oligonucleotide; and the other of said first or second oligonucleotides comprises a primer or a second promoter-primer,
one or more DNA and/or RNA dependent DNA polymerases, and
an RNA polymerase that recognizes a promoter within one or both of said first or second promoter-primers.

25. The composition of claim 24 wherein said target is RNA.

26. The composition of claim 24 wherein said target is DNA and wherein said first oligonucleotide hybridizes to said target distant from the 3' end of nucleic acid comprising said target.

27. The composition of claim 24 wherein said composition further comprises RNase H activity.

28. The composition of claim 27 wherein said RNase H activity is supplied by an exogenous RNase H from E. coli.

29. The composition of claim 24 or 27 wherein a reverse transcriptase comprises both said DNA-dependent DNA polymerase and said RNA-dependent DNA polymerase.

30. The composition of claim 29 wherein said reverse transcriptase further comprises said RNase H activity.

31. The composition of claim 24 wherein both of said first and second oligonucleotides comprise promoter-primers, each having a promoter recognized by said RNA polymerase.

5 32. The composition of claim 24 further comprising one or more of DMSO, dimethylformamide, ethylene glycol, zinc and glycerol.

Sub H3 33. The composition of claim 24 wherein said mixture allows amplification at essentially constant temperature.

10 34. The composition of claim 24 further comprising one or more helper oligonucleotides.

35. A kit comprising the following components:
a first and a second oligonucleotide of opposite sense, one of said first or second oligonucleotides able to
15 complex at or near the 3'-end of a target nucleic acid sequence and the other of said first or second oligonucleotides able to complex at or near a 3'-end of a nucleic acid sequence complementary to said target nucleic acid sequence, wherein one of said first or second
20 oligonucleotides comprises a first promoter-primer and consists essentially of a single nucleic acid sequence having both modified and unmodified members or a mixture of differently modified members, and the other of said first or second oligonucleotides comprises a primer or a second
25 promoter-primer, wherein said modified member is modified to reduce extension of said oligonucleotide by a polymerase compared to an unmodified oligonucleotide;
one or more DNA and/or RNA dependent DNA polymerases,
and

~~an RNA polymerase that recognizes a promoter within one or both of said first or second promoter-primers.~~

36. The kit of claim 35 further comprising an exogenous RNase H.

5 37. The kit of claim 35 further comprising one or more helper oligonucleotides.

38. The kit of claim 35 further comprising one or more probes able to indicate the presence of said target ribonucleic acid, or its complement.

10 39. A kit containing two oligonucleotides each consisting essentially of a single nucleic acid sequence selected from the group consisting of
xGCCGTCACCCACCAACAAGCT, xGGGATAAGCCTGGGAAACTGGGTCTAATACC,
xCCAGGCCACTTCCGCTAACC, and xCGCGGAACAGGCTAAACCGCACGC,
15 wherein x is nothing or is a sequence recognized by an enzyme.

40. An oligonucleotide consisting essentially of a single nucleic acid sequence and selected from the group consisting of xGCCGTCACCCACCAACAAGCT,
20 xGGGATAAGCCTGGGAAACTGGGTCTAATACC, xCCAGGCCACTTCCGCTAACC, and xCGCGGAACAGGCTAAACCGCACGC, or an oligonucleotide complementary to any one of said single nucleic acid sequences, wherein x is nothing or is a sequence recognized by an enzyme.

25 41. A kit containing oligonucleotides consisting essentially of the following sequences:
xGCCGTCACCCACCAACAAGCT, xGGGATAAGCCTGGGAAACTGGGTCTAATACC,

and GTCTTGTGGTGGAAAGCGCTTTAG, wherein x is nothing or is a sequence recognized by an enzyme.

42. A kit containing oligonucleotides consisting essentially of the following sequences:

5 xCCAGGCCACTTCCGCTAACC, xCGCGGAACAGGCTAAACCGCACGC, and GGAGGATATGTCTCAGCGCTACC, wherein x is nothing or is a sequence recognized by an enzyme.

43. A method for amplifying Mycobacterium nucleic acid in a sample comprising amplification of said nucleic acid with one or more oligonucleotides consisting essentially of a single nucleic acid sequence and selected from the group consisting of xGCCGTCACCCACCAACAAGCT, xGGGATAAGCCTGGGAAACTGGGTCTAATACC, xCCAGGCCACTTCCGCTAACC, and xCGCGGAACAGGCTAAACCGCACGC, wherein x is nothing or is a
15 sequence recognized by an enzyme.

44. A method for detection of M. tuberculosis nucleic acid in a sample comprising hybridization of nucleic acid derived from said sample with an oligonucleotide consisting essentially of a single sequence and is
20 selected from the group consisting of GTCTTGTGGTGGAAAGCGCTTTAG and xCCAGGCCACTTCCGCTAACC.

45. A method for the detection of M. tuberculosis nucleic acid comprising amplification of said nucleic acid with one or more oligonucleotide polymers consisting essentially of the sequences xGCCGTCACCCACCAACAAGCT, and xGGGATAAGCCTGGGAAACTGGGTCTAATACC and detection of the amplified nucleic acid with a nucleotide polymer consisting essentially of the sequence GTCTTGTGGTGGAAAGCGCTTTAG,

wherein x is nothing or is a sequence recognized by an enzyme.

46. A method for the detection of M. tuberculosis nucleic acid comprising amplification of said nucleic acid with one or more oligonucleotide polymers consisting essentially of the sequence xCCAGGCCACTTCCGCTAACC and xCGCGGAACAGGCTAAACCGCACGC, and detection of the amplified nucleic acid with a nucleotide polymer consisting essentially of the sequence GGAGGATATGCTCTCAGCGCTACC, wherein x is nothing or is a sequence recognized by an enzyme.

47. The method of claim 43, 45 or 46 wherein said enzyme is an RNA polymerase.

Sub B2 48. The kit of claim 41 or 42 wherein one or more of said sequences has a modified 3'-end.

15 49. The kit of claim 41 or 42 comprising a mixture comprising modified and unmodified members comprising one or more of said sequences.

20 50. The oligonucleotide of claim 40 wherein said sequence, or said oligonucleotide complementary thereto, has a modification at its 3'-end.

Sub B2 51. The oligonucleotide of claim 40 comprising a mixture comprising modified and unmodified members or differently modified members comprising said sequence, or said oligonucleotide complementary thereto.

25 52. The method of claim 43, 45 or 46 wherein one or more of said sequences has a modified 3'-end.

53. The method of claim 43, 45 or 46, comprising a mixture comprising modified and unmodified members comprising one or more of said sequences.

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